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Nephrotoxicity induced by titanium dioxide nanoparticles (TiO2 NPs) in albino mice and the possible protective role of vitamin E (A histological and molecular study)

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ABSTRACT **: TiO2 NPs are used in many fields such as food industry, medicine and agriculture. Despite the advantages of nanotechnology, the adverse effects of nanoparticles are inescapable. The current study was designed to assess the protective impact of vitamin E (VE) on the nephrotoxicity of TiO2 NPs at the histological and molecular level. Mice were randomly divided into 4 groups: G 1, control without any treatment; G 2, vitamin E (100 mg/kg/day); G 3, mice exposed to TiO2NPs (1499 mg/kg/day) for 5 consecutive days ; G4, TiO2-NPs+VE daily. Histologically, TiO2-exposed animals revealed highly-destructed renal parenchyma with ruptured glomeruli, apoptotic nuclei, leucocytic infiltration, damaged distal convoluted tubules containing proteinaceous material in its lumens, highly distorted glomerular elements and focal necrosis. Molecularly, TiO2 NPs-intoxicated animals resulted in a marked increase in DNA fragmentation for 2.4 folds in tail DNA (2.404 %) and 4.9 folds in tail moment DNA (7.093) as compared to control. Co-administration of vitamin E to TiO2 NPs-intoxicated mice partially protected their renal tissue from DNA damage as indicated by a marked decrease in tail DNA (2.049%) and tail moment (5.347) as compared to that given TiO2 NPs only. However, VE co-treatment with TiO2 nanoparticles partially-returned the renal tissue to the normal histological structure and minimized DNA damage, where a mild degree of cellular repair in the renal parenchyma could be detected. We can conclude that VE supplementation may play a promising role in reducing the nephrotoxicity of TiO2 NPs in experimental animals at the level of applied doses.**

KEYWORDS: Titanium dioxide, kidney, albino mice, histopathology, comet assay.

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I. INRODUCTION

The increasing use of nanoparticles in different aspects of life (medicine and food industry) increases chronic exposure to titanium particles. Toxicity of nanoparticles (NPs) is an important issue to be studied since NPs are widely-used in a vast range of biomedical applications, mainly in drug delivery, in health and diseases (Zahin et al. 2020). Previous studies have recorded damage to different organs such as testis, lungs, heart, brain and others as affected by NPs exposure (Nassar et al., 2017; Nassar et al., 2018; Warheit and Brown, 2019; Nassar, Salma et al., 2021). Titanium dioxide nanoparticles (TiO2 NPs) are ultrafine particles of tiny sizes ranging from 1 to 100 nanometers and could accumulate in kidney and liver inducing nephrotoxicity and hepatotoxicity in male rats (Morgan et al., 2017). Since kidneys are responsible for excreting waste products from blood and regulating metabolic water, they are the major target of TiO2 NPs deposition and toxicity even in low level of TiO2 NPs exposure (Wang et al., 2007; Liu et al., 2009; Chen et al., 2009; Zhao et al., 2010). Accumulation of TiO2 NPs in different organs of experimental animals (kidneys, liver, spleen, lymph node, heart, and lungs) could be documented and could not be cleared from the liver and kidney before 15 days after

administration (Li and Chen, 2011).The toxicity of TiO 2NPs can be induced by several mechanisms. These small particles could induce genetic toxicity through changing gene expression (Geiser et al., 2005; Ma et al., 2009). Natural products with their bioactivities and pharmaceutical potentials are used as drugs and medicines (Lamottke et al. 2011). Vitamins are good antioxidants to protect tissues from oxidative stress. Vitamin E comprises eight natural fat soluble compounds, including 4 tocopherols (alpha, beta, gamma and delta) (Diab et al., 2015).

Assessment of the toxicity risk is a complex process to balance the risk and benefits of using such nanomaterials. So, we must find ways to reduce the impact of nanoparticles, especially those used in the food industry. Therefore, the present investigation was planned to assess the protective impact of vitamin E (VE) on the nephrotoxicity of TiO2 NPs at the histological and molecular level.

2. MATERIALS AND METHODS

Chemicals:

Titanium dioxide (TiO2 NPs - anatase form), Vitamin E, and all other chemicals used in the current experiment were of the highest analytical grade available and purchased from Sigma-Aldrich Co. Preparations: TiO2 NPs powder was suspended in bi-distilled water at a dose of 1944 mg / kg for IP injection in our experiment.
Experimental setup:

Animals (40 adult male albino mice) were distributed into 4 groups (10 mice/group) as follows: G 1, control animals which fed on a basal diet and water without any treatment; G 2, vitamin E-given mice (100 mg/kg/day); G 3, mice exposed to a dose of an aqueous solution of TiO2NPs (1499 mg/kg/day, i.p) for 5 consecutive days (Shukla et al., 2011). G4, TiO2-NPs+VE daily. Vitamin E was orally- administered at a dose level of 100 mg/kg/day (Sharma et al., 2003) for 7 consecutive days, two of them before the start of TiO2 NPs administration. After the last dose administration, mice were sacrificed. Kidney specimens of control and treated animals were extracted and a number of them was fixed in 10% neutral buffered formalin at room temperature for histopathological examination while others were kept in a freezer for a molecular (comet assay) study.

Histopathological Study:

Kidney specimens were dehydrated, cleared and embedded in paraffin wax. Paraffin blocks were sectioned at 3-4 µm, stained with hematoxylin and eosin (Bancroft and Gamble, 2002), and finally examined by light microscopy.

Molecular Study:

Comet Assay:

Comet, or single cell gel electrophoresis (SCGE), is an accurate technic for measuring DNA fragmentation in separate individual cells in alkaline medium (Singh et al., 1988). Finally, the program calculates the tail moment by multipling the % of DNA in the tail by the tail length (Olive et al., 1990). Analyze from 50 to 100 randomly-selected cells per each sample.

3. RESULTS

3.1 Histopathological observations

Examination of H&E-stained sections of control animals, by the light microscope, exhibited normal histological structure of their kidney (Fig. 1). Mice of VE group revealed no histological variations from that of control ones (Fig. 2). However, exposure of experimental animals to TiO2 NPs (1.944g/kg/day) for five consecutive days revealed several histopathological alterations in the renal tissue. The changes included highly-destructed renal parenchyma with ruptured glomerular elements that appeared containing apoptotic nuclei and degenerated proximal and distal convoluted tubules (Fig. 3). Further examination of another fields of the renal tissue of TiO2-exposed animals detected inflammatory leucocytic infiltration in the renal parenchyma, damaged distal convoluted tubules containing proteinaceous material in its lumens, highly distorted glomerular elements and focal necrosis (Fig. 4). However, VE co-treatment with TiO2 nanoparticles partially-returned the renal tissue to the normal histological structure where a mild degree of cellular repair in the cortical parenchyma could be detected. The co-administration of VE together with TiO2 potentiates a good phase of regeneration in the histology of renal cortex. The glomerulus, proximal and distal convoluted tubules appeared well-organized with a nearly normal cellular constituents. But some apoptotic nuclei and glomerular shrinkage are still present (Fig. 5).

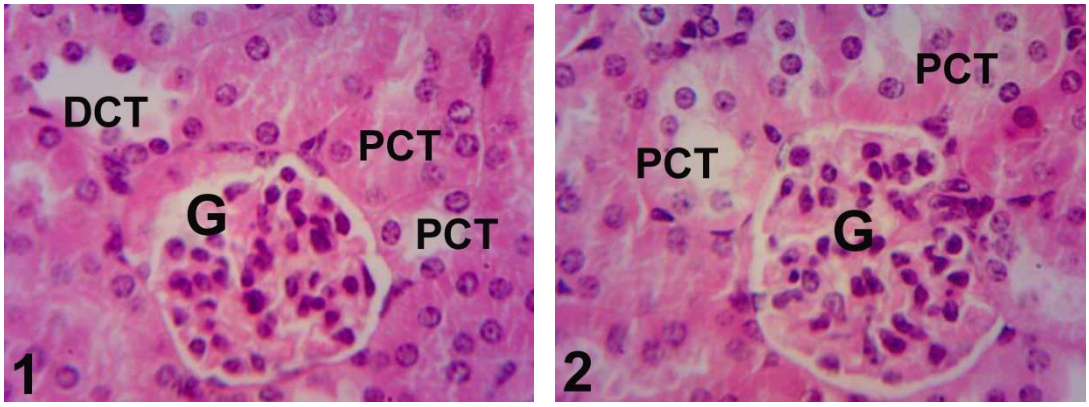


Fig. 1: Section of kidney cortex of control mice (H and E, X1000) illustrating the normal histological structure of kidney including glomerulus (G) with intact parietal layer of Bowman's capsule (arrow), normal proximal and distal convoluted tubules (PCT, DCT).

Fig. 2: Section of kidney cortex of VE-administered mice (H and E, X1000) showing no histological changes with renal parenchyma similar to that of control. G: glomerulus, PCT: proximal convoluted tubule, DCT: distal convoluted tubules.

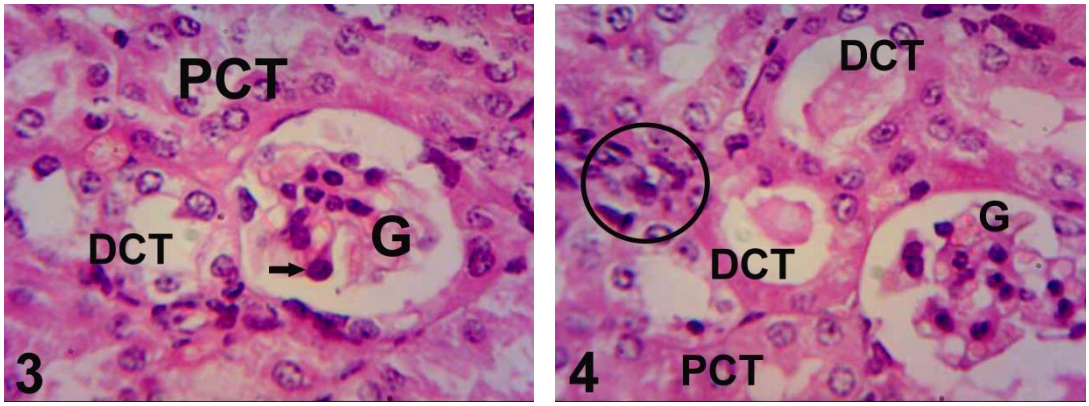


Fig. 3: Section of kidney cortex of Tio2-exposed mice (H and E, X1000) exhibiting highly-degenerated renal parenchyma with ruptured glomerular elements (G) containing apoptotic nuclei (arrow) and damaged proximal and distal convoluted tubules (PCT, DCT).
Fig. 4: Section of another field of kidney cortex of Tio2-exposed mice (H and E, X1000) showing leucocytic infiltration in the renal parenchyma (circle), damaged DCTs containing proteinaceous material (arrows), highly distorted glomerulus (G) and necrotic areas (N).

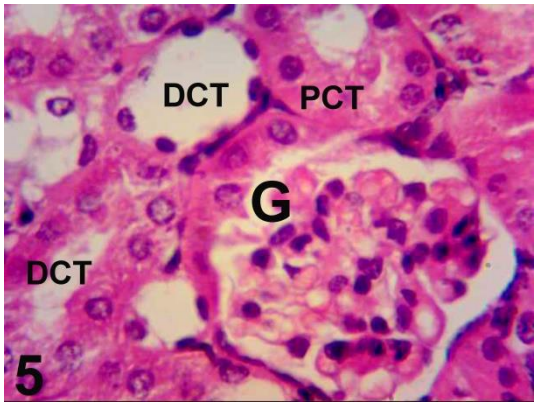
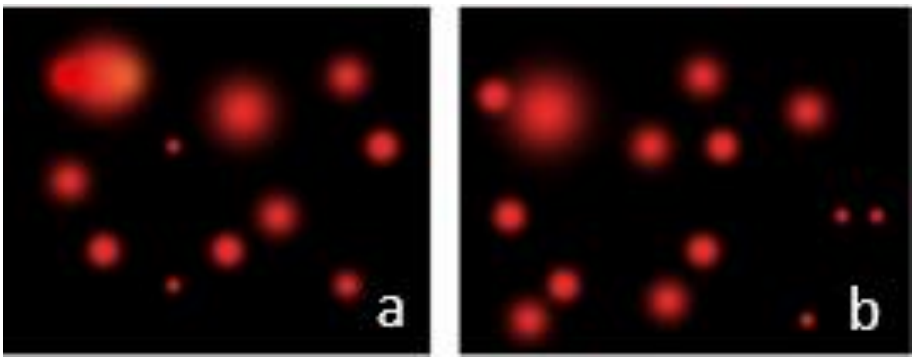


Fig. 5: Section of kidney cortex of TiO2-exposed mice treated with VE (H and E, X1000) exhibiting a mild degree of cellular repair in the cortical parenchyma due to pre and co-treatment with VE, where glomerulus (G), PCT and DCT are partially-restored their histological structure. But some apoptotic nuclei and glomerular shrinkage are still present.

3.2. Molecular observations

Comet assay for DNA Fragmentation:

The application of Comet assay technology on renal tissue of control and experimental mice revealed varying degrees of DNA damage and confirmed the preceding histopathological results. DNA fragmentation of control animals was; a tail DNA 1.114 % and a tail moment of 1.459 (Fig.6-a, Table 1). As shown in table 2 no any statistical significant difference was observed in group treated with vitamin E as compared to those of control, where tail DNA was 1.071% and tail moment was 1.349 (Fig.6-b, Table 1). Exposure of experimental animals to TiO_2 NPs resulted in a marked increase in DNA fragmentation for 2.4 folds in tail DNA (2.404 %) and 4.9 folds in tail moment DNA (7.093) (Fig.6-c, Table 1). Co-administration of vitamin E to TiO2 NPs-intoxicated mice partially protected their renal tissue from DNA damage as indicated by a marked decrease in tail DNA (2.049%) and tail moment (5.347) as compared to that given TiO_2 NPs only (Fig.6-d,Table 1).



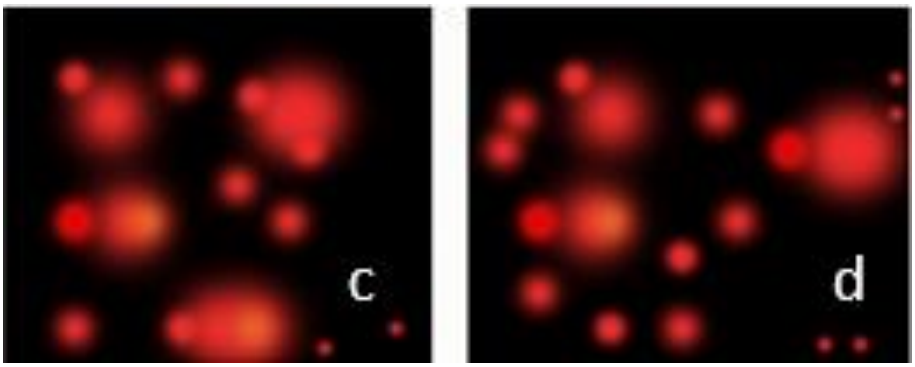


Fig. 6: Comet assay exhibiting varying degrees of DNA damage in the renal tissue of TiO2-NPs-intoxicated mice and the effect of vitamin E treatment: (a) Control mice, (b) VE group, (c) TiO2-NPs-intoxicated group (d)TiO2-NPs+VE group and treated with vitamin E.

Table.1: Values of Comet parameters in control and experimental mice

	Tailed	Untailed	Tail Length	Tail DNA	Tail moment
	%	%	µm	%	Units
NC	4	96	1.31	1.114	1.459
VE	4	96	1.26	1.071	1.349
TiO_2	11	89	2.95	2.404	7.093
TiO_2+VE	8	92	2.61	2.049	5.347

4. Discussion

The results of the current biological study showed that TiO2-exposed animals revealed highly-destructed renal parenchyma with damaged glomeruli, apoptotic nuclei, leucocytic infiltration, damaged renal tubules and focal necrosis. Molecularly, TiO2 NPs-intoxicated animals resulted in a marked increase in DNA fragmentation as compared to control. The authors attributed these changes to the oxidative stress exerted by TiO2 NPs and their ability to combine with macromolecules as proteins, lipids and DNA. However, co-administration of TiO2NPs plus VE, in the present experiment, could improve the histological pattern of kidney and minimize DNA fragmentation. These results confirm and extend comparable result in the heart tissue in a first part of the same study for the same authors published in (2021) which verified the cardiotoxicity of TiO2 NPs and documented the protective role of VE against harmful effects of nanomaterials (Nassar et al., 2021). The current results are in agreement with a number of previous studies; TiO2 NPs exert their effect in three mechanisms, ROS production which inducing oxidative stress and attachment to cell membranes, intracellular organelles and macromolecules causing damage and lipid peroxidation (Hou et al ., 2019). The large surface area of TiO2NPs enhances their capacity to produce ROS that cause cytotoxicity and genotoxicity. Moreover, the accumulation of ROS induces oxidative damage of macromolecules such as lipid, protein, and DNA peroxidation and alterations in gene expression (Erma and Davies, 2002). The selective nephrotoxicity of TiO2 NPs have been proved where TiO2 NPs could be accumulated in kidneys of rainbow trout and the molecular mechanisms were responsible for this toxicity (Scown et al., 2009). Kidneys are more susceptible to toxic materials due to its high blood supply (it receives 20–25% of the cardiac output) and concerned with elimination of harmful substances; therefore, kidneys are considered to be one of the vital organs vulnerable to the effect of TiO2(Pujalté et al., 2011). Kidney and liver are also organs of high energy turnover. Intravenously applied nano-TiO2 (10–20 nm) caused massive oxidative

stress, DNA damage, and mitochondria-mediated apoptosis in the kidneys and liver of rats. (Meena and Paulraj. 2018). Previous studies, also, reported that the action of TiO2 induced nephrotoxic effect was mediated via release of the products of oxidative stress such as cytokines, reactive oxygen species and at the same time the decrease of cellular antioxidants. These ROS are injurious to cell components as lipid, protein and inactivate structural proteins, enzymes, and ion pumps, increase lipid peroxidation, inflammation, cytolysis, interstitial fibrosis, mutation and damage to DNA and apoptosis(Oberdörster et al., 2013; Grande and Tucci, 2016). TiO2 nanoparticles generate free radicals, which induced indirect genotoxicity mainly by DNA-adduct formation. The rutile-based Nano-TiO2, as a result of photocatalysis, produced reactive oxygen species (ROS), then causing DNA damage in vitro(Brezová et al., 2005; Bhattacharya et al., 2009). Bee bread ethanolic extract provides a protective effect against the metabolic and histological disorders induced by the subacute intoxication of rats with TiO2 nanoparticles. The histological analysis of the kidney tissue showed that TiO2 induced congestion when administered alone However, the coadministration of bee bread extract with TiO2 for 30 days has reduced the intensity of congestion in renal tissue (Bakour et al., 2021). Kidneys, exerting the function of eleminating xenobiotics, showed oxidative stress, increased inflammation markers, as well as functional and histological damages due to tubular necrosis in rats treated orally with (unspecified) nano-TiO2. (Fadda et al., 2018). In another work, lipid peroxidation, reduced antioxidant activity, and proximal tubular apoptosis has been observed in the kidneys of rats after oral gavage of TiO2 NPs for 3 weeks (Alidadi et al., 2018). Organs of high energy demand, such as brain or kidneys, show special sensitivity to oxidative stress because of their intense mitochondrial activity. (Guerra-Araiza et al., 2013; Gyurászová et al., 2020). TiO2NPs (28.9 nm and zeta potential of −33.97 mV) induced several toxicological and the pathological changes in kidney, liver, and testis as indicated by the disturbances in different biochemical parameters, oxidative stress markers. TiO2NPs also induced elevation of DNA fragmentation and chromosomal aberration in somatic and germ cells as well as the increased number of abnormal sperms. Cinnamon oil may be used as a safe candidate for the protection against TiO2NPs. On contrary to our results, Basante-Romo et al. (2021) reported that the application of modified-TiO2 NPs (a novel photocatalytic nanomaterial, in doses equal to or less than 5,000 mg/kg b.w, i.p) for the first time, in vivo, revealing that m-TiO2 NPs administered i.p. in albino CFW mice does not have any toxic effect. The authors conclude that m-TiO2NPs is safe and practically non-toxic when injected i.p. Therefore, we can conclude that VE supplementation exerted a protective role in minimizing the confirmed nephrotoxicity of TiO2 NPs in experimental animals at the level of the applied doses. Further studies are needed to know the exact protection mechanism and the active ingredient responsible for these beneficial effects.

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